



Department of Pesticide Regulation
Environmental Monitoring Branch
Attn: Bill Fabre
1001 "I" Street
PO Box 4015
Sacramento, CA 95812-4015
(916) 324-4191

June 26, 2002

Dear Bill,

Re: Contract Agreement #01-0183C between the Department of Pesticide Regulation and the Foundation of California State University Monterey Bay.

The Central Coast Watershed Studies (CCoWS) team of the Watershed Institute, California State University Monterey Bay (CSUMB), has completed staff training and lab augmentations necessary for the monitoring of Diazinon and Chlorpyrifos in nine impaired water bodies of the lower Salinas region.

Please find enclosed the following deliverables in relation to the above contract:

Deliverable 1. Study area description

A description of the study area has been completed and is included in the accompanying draft Report No. WI-2002-06. This status report will eventually become the final project report as subsequent sections are added. It provides background, aims & general methodology, previous work and describes the study area. Included in the study area description is surrounding land-use in the proximity of the sampling locations with narratives on distances to key points of interest and sites relative to each other. Included is a map of the study area and a picture taken at each sampling site.

Deliverable 2. ELISA methodology, field and lab QA/QC protocols

The accompanying report, WI-2002-05c: Protocols for Water Quality and Stream Ecology Research, contains standard procedures for CCoWS management, field, and laboratory operations (dated June 25, 2002). It is a general document covering all aspects of research conducted by CCoWS. It is a dynamic operations manual that is constantly evolving as techniques and methodologies are improved upon or added. Sections that directly address this milestone's deliverables are:

- Section 3.13: Protocol for sampling pesticides
- Section 4.5: Protocol for analyzing pesticide samples

These sections summarize the ELISA methodology and QA/QC protocols for field collection and laboratory analysis.

Deliverable 3. Summary of training

This deliverable is outlined as follows:

Enzyme Linked ImmunoSorbent Assay (ELISA) has proven to be a useful, cost-effective way to obtain rapid information on Diazinon and Chlorpyrifos concentrations in environmental water samples. Additionally, techniques for pesticide extraction from sediment have been developed. Therefore, ELISA can also be used to determine pesticide concentrations within sediment. For these reasons, it was decided to augment our existing water quality analytical capabilities with the ELISA technology.

In relation to the present project plan, the following steps were taken:

- Reviewed literature, including but not limited to:
 - ELISA measurement of Diazinon in water and sediment: principles and operating procedures (Katznelson & Feng, 1998). A key document for the development of our laboratory protocols, it was reviewed extensively and questions concerning content and technique were addressed directly to the author, Dr. Katznelson.
 - Filtration of water-sediment samples for the determination of organic compounds (Sandstrom, 1995)
 - Patterns of aquatic toxicity in an agriculturally dominated coastal watershed in California (Hunt et al., 1999)
 - Diazinon in surface waters in the San Francisco Bay area: occurrence and potential impact (Katznelson & Mumley, 1997)
 - Instructional materials from ELISA kit manufacturers
- Consulted experts:
 - Dr. Revital Katznelson, State Water Resources Control Board, has visited our lab twice and assisted us in improving the accuracy of our methods. She has included CCoWS in a network of ELISA labs found in California.
 - Dr. John Hunt and Brian Anderson, Marine Pollution Studies Lab
 - Dr. Sharon Anderson, director of the Earth Systems Science and Policy program at CSUMB, professor of Chemistry
 - Margaret Geissler, Cheri Everlove and Kendell Silveira, lab director and technicians at CSUMB
 - Technicians at the Monterey County Consolidated Chemistry Laboratory demonstrated to us their procedures for the general ELISA technique.
- Purchased equipment:
 - Micro-dispensers
 - Various pipettes
 - Micro-well strip reader
 - Incubator
- Tested ELISA kits:
 - Purchased 4 Diazinon and 3 Chlorpyrifos kits
 - Performed spike and dilution tests
 - Performed ELISA runs on actual environmental samples
 - Compare ELISA results to that of GC/MS (pending)

Seven ELISA kits, containing 96 wells per kit, have been used throughout the training process. Correlation coefficients obtained by our calibration curves have ranged from 0.82 during our beginning runs to 0.99 for our later runs, with our current abilities consistently achieving 0.95 or higher. Only 2 of our last 23 calibration pairs had a percent coefficient of variance (%CV) over 15%. Spikes have been recovered with a relative percent difference (RPD) of 6%; dilutions have been performed with a 22% error. The average percent %CV during our last run was 5% for all replicates. Our next step (in progress) is a comparison of values our ELISA technique obtains to that of an outside lab using GC/MS. In summary, the training

process has resulted in strong capability for stable and robust analysis of Diazinon and Chlorpyrifos using ELISA technology.

The senior technician on the project, Don Kozlowski, has trained two student technicians on the ELISA analysis technique. He has also trained the field manager and 4 technicians on the field sampling technique. We are prepared to proceed with the first monitoring run and analysis in July.

Please let us know if anything further is required.

Sincerely,

Don Kozlowski, Senior Technician

Dr. Fred Watson, Project Leader